NEUROLOGICAL AND PHYSIOLOGICAL RESPONSES OF THE PRIMATE TO ANTHRAX INFECTION*

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Anthrax toxin has been shown by Vick et al (1968) to cause several major physiological changes in the rhesus monkey following toxin challenge. They showed that there was a partial depression or complete loss in cortical electrical activity (EEG) which occurred as early as 5 minutes after toxin administration, followed in some cases by depression in cyclic patterns that appeared independent of other physiological changes. A few hours before death increased depression was followed by anoxic hypertension that appeared to progress with the degree of intoxication to final cardiovascular collapse. During this progressive intoxication acute respiratory difficulties became apparent, ultimately terminating in apnea. Respiratory paralysis and the resulting death of the monkeys were attributed to the direct action of the anthrax toxin in the deep respiratory center of the brain, since conductance over the phrenic nerve and neuromuscular junction was shown to be intact. These physiological changes and death by intoxication could be prevented if antitoxin was administered before or within 8 hours after injection.

Responses of the toxin-injected monkey prompted this parallel study with the infection itself. A remarkable similarity between responses of the bacterial disease and the toxin-challenged animals was observed.

METHODS

The rhesus monkeys (*Macaca mulatta*) used were from Pakistan and weighed 4 to 5 kg. The 5 animals in the group on which EEG were recorded had electrodes permanently implanted on the dura of the brain by standard techniques at least 21 days before challenge. Electrical cortical activity was recorded on a model III D Grass Polygraph (Grass Instruments, Quincy, Massachusetts). Another group of 6 animals was used for simultaneous recordings with a Sanborn Model 964-1100 (Sanborn Co., Waltham, Massachusetts) for respiratory rates and electrocardiograms (EKG). Hematocrit values, white blood cell counts, number of bacilli, and units of toxin per ml of blood were taken on an 8-hour schedule after challenge until death. Constant observation was exercised, and additional samples were obtained as the time of death approached and at death. Because of electrical interference, we were unable to record both EEG and EKG on the same animal. The methods for monitoring neurological and physiological responses are those of Vick et al (1968) and Klein et al (1966), respectively.

Received for publication June 10, 1967.

We wish to extend our thanks to Col. W. R. Beisel, Fort Detrick, for his comments and encouragement in the preparation of this manuscript.

* In conducting the research reported herein, the investigators adhered to "Guide for Laboratory Animal Facilities and Care" established by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

Animals were challenged intradermally with 1×10^6 spores of the Vollum (VIb) strain of *Bacillus anthracis*.

RESULTS

Septicemia and blood cell changes. Individual populations of organisms in the blood of the 6 monkeys infected with anthrax are summarized in figure 1.

The dose of 10^6 spores, which is 2 to 10 times the dose used in work reported earlier (Klein et al, 1966), resulted in times to death of 32 to 62 hours. This time is roughly twice that at which monkeys died following challenge with 10,000 units of anthrax toxin (Vick et al, 1968). In all animals septicemia was observed at the 24 to 30-hour sampling period and increased until death. Toxin was not detected until the terminal sample. Its level varied between 15 and 110 rat units (Haines et al, 1965) and was demonstrated in all animals.

The white blood cell counts and the hematocrit levels paralleled the more extensive data published earlier by Kline et al (1966). There was an absolute leukocytosis, and the hematocrit reading increased in 4 of 6 animals; the other 2 remained within normal range.

Electroencephalography. The EEG trace for 1 animal is shown in figure 2. It is typical for the group on which tracings were obtained, with additional observations noted below. This animal died at 62 hours, 7 minutes. The terminal number of bacilli per ml of blood was 10^5, a number somewhat lower than usually found.

The tracing before challenge was normal for the monkeys we have observed. An incipient septicemia occurred between the sampling periods of 16 and 22 hours, so that at 22 hours 10^2 bacilli per ml were observed. The EEG remained essentially normal. At 44 hours the organism level in the blood had increased to 10^4 organisms per ml, which was just at the detectable level by direct microscopic observation. The first major change occurred at 54 hours when EEG tracings showed signs of depressed electrical cortical activity. From 54 to 62 hours (7 minutes before respiration ceased) the EEG tracings showed that electrical cortical activity steadily declined to zero. This loss of cortical activity paralleled the observations of Vick et al (1968) on monkeys injected with sterile anthrax toxin.

Electrocardiography and respiration. The recordings of 1 monkey are given as typical for the group (figure 3). Each monkey was used as his own control prior to challenge. Both cardiac and respiratory rates were within the normal range (heart rate, 192 per minute; respiration, 40 per minute). This representative monkey died at 56 hours, 36 minutes with 10^8 organisms per ml of blood. The septicemia of 10^4 organisms per ml was first noticed at 24 hours and steadily increased during the 32 hours prior to death.

With the onset of septicemia at 24 hours dyspnea was definitely noticeable and became more severe as the disease progressed. Except for a short period at 51 hours when it dropped to 25 per minute, the respiratory rate remained constant (45 ± 3) until 3 minutes before death, when it gradually fell to zero.

Through this entire period until 16 minutes before death, the EKG remained essentially normal. Although the heart rate showed some variation (250 to 150 per minute), it stayed within normal limits. In the last 16 minutes the EKG amplitude, along with the heart rate, decreased rapidly and uniformly. A heart beat was detected for 5 minutes after respiration ceased, an observation consistent with that of either rats or monkeys killed with sterile anthrax toxin.

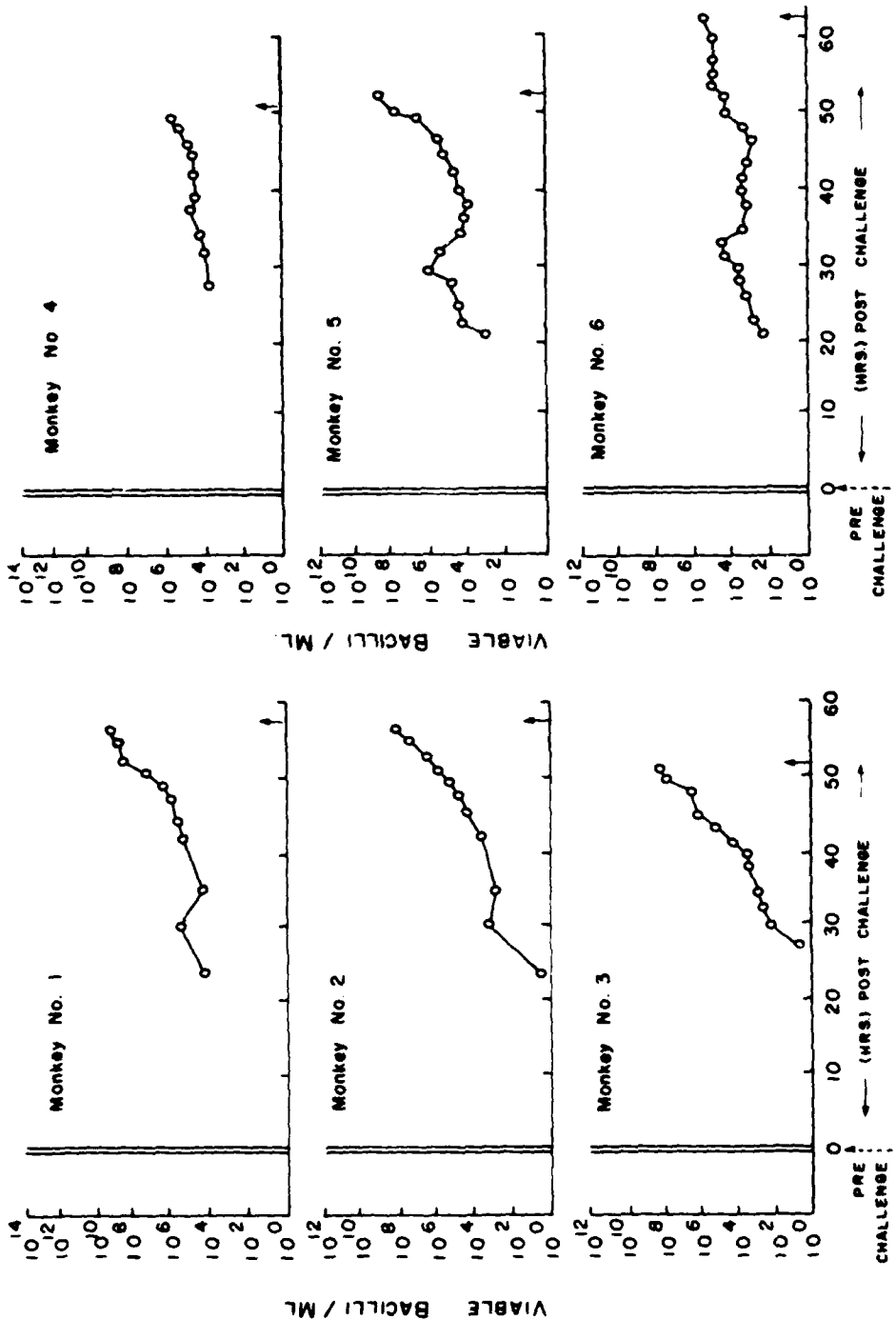

Fig. 1.—Observations of anthrax septicemia in rhesus monkeys.

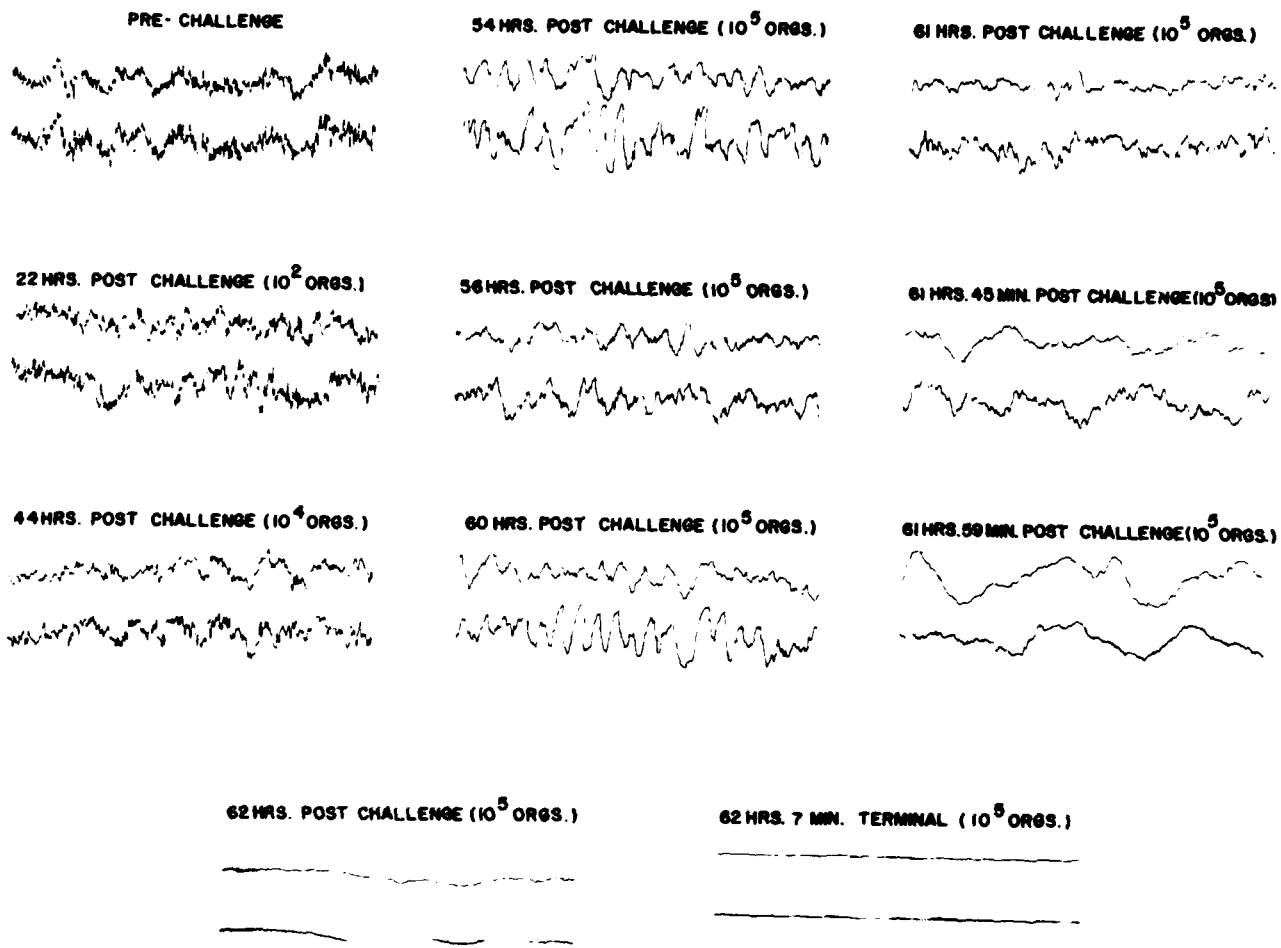

FIGURE 2. EEG tracings of the rhesus monkey before challenge and during lethal anthrax infection.

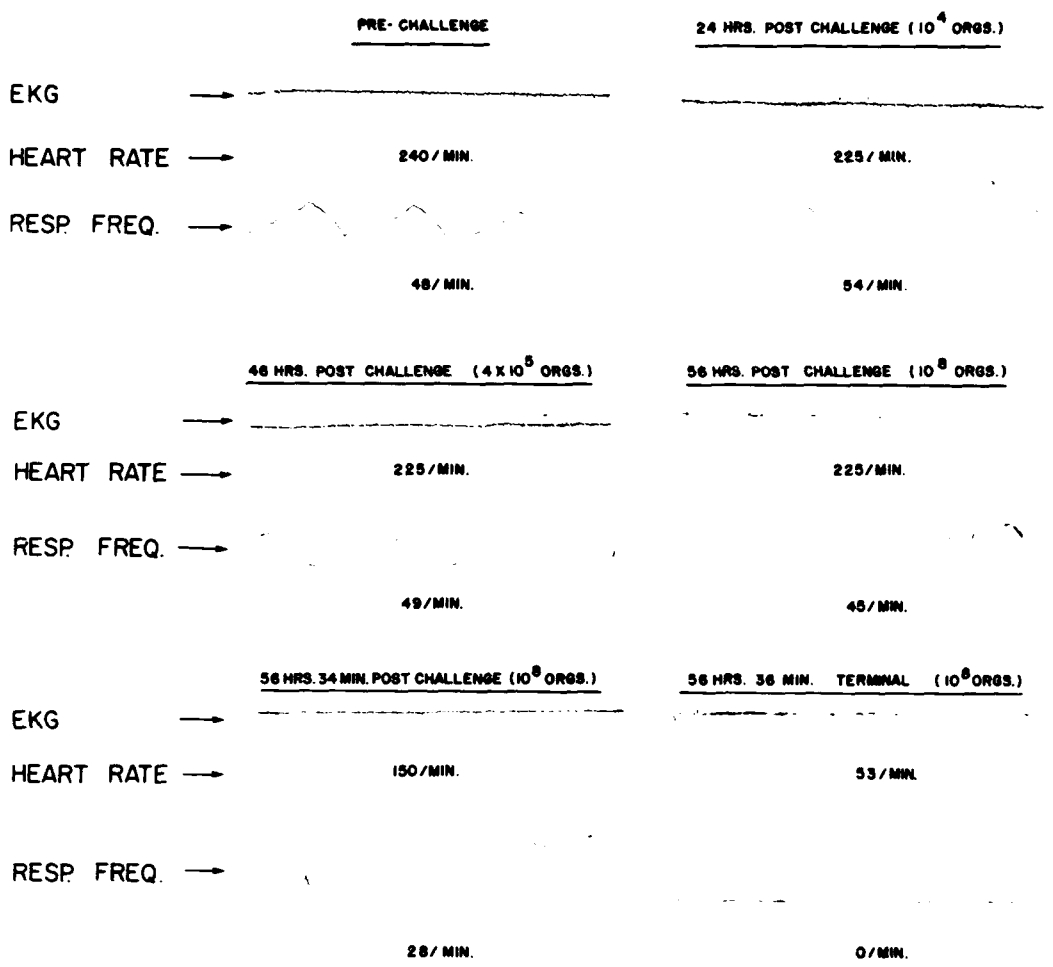

FIGURE 3. Respiratory and cardiovascular responses in the rhesus monkey during anthrax infection.

DISCUSSION

After sufficient organism growth in the host lethal quantities of toxin are produced, and anthrax becomes essentially a toxigenic disease, leading to a disease syndrome identical to that seen with sterile anthrax toxin. The difference in time of appearance of signs is attributable to the fact that in the spore-induced disease toxin builds up slowly in some direct relationship to the number of bacilli present in the body; with the toxin challenge a lethal dose is given over a short period of time. The signs in the last half of life for both the disease and toxin challenge are remarkably comparable and parallel. The central nervous system is progressively depressed, but the cardiovascular system functions normally until preterminal apnea. Some degree of dyspnea is observed but, preterminally, respiration becomes quite abnormal and stops. Parallel with and following respiratory failure cardiovascular changes become increasingly evident until cardiac arrest.

Middleton and Standen (1961), working with 2 monkeys (we exclude 1 with an *E. coli* coinfection and 1 that was sacrificed), observed an intact myocardium and normal EKG during the course of disease. A recording on an animal made at 2 hours before death indicated no significant change in rate or function. Apparently no changes were observed in either of the animals at approximately 18 hours before death. However, recordings were not made near death of the remaining monkey, and time to death was not observed or known within 5 or 6 hours. Free toxin was demonstrated in terminal blood of both monkeys tested. We agree with their remark that "It is difficult to reconcile the tracings of a remarkably intact myocardium with the clinical picture of an overwhelming bacteremia in a moribund animal." However, we disagree with their conclusion that "circulatory shock in anthrax probably results from toxin damage to the peripheral tissue, causing loss of vascular tone and integrity." This statement is not consistent with and supported by their data. Our work shows that cardiovascular changes follow respiratory changes, an observation overlooked or ignored by these and other workers.

The correlation between the physiological parameters recorded in our work and in field and laboratory observations on anthrax cases is quite consistent. Except for a few cases of dullness, depression, and anoxia, infected animals showed few signs until death suddenly occurred. The literature on anthrax, whether for humans or animals, repeatedly uses terms that mean unexpected, sudden death. We are especially appreciative of the observations of Mansjoer (1961) on Javanese humans. He states that ". . . suddenly unexpected death occurred even though a few hours earlier the patient had talked calmly and showed no sign of grave illness. A few minutes before death, the patient became cyanotic . . . ." Pienaar (1961), reporting on African veldt animals, writes, "In the majority of cases it appeared that death occurred suddenly and there were few or no signs of kicking and struggling. A zebra was found dead with a mouthful of green grass on which it was feeding. In other cases death set in more gradually and these cases could not be referred to the peracute form of the disease." There is nothing in our results and very little in records of field cases to indicate that slow death and complications occur, such as would happen if the kidneys were shut down, resulting in "secondary shock" (Smith et al, 1955), or if pulmonary edema occurred (Beall and Dalldorf, 1966), resulting in hemoconcentration and a prolonged struggle for oxygen with extended death agony.

As with every complex biological phenomenon there are exceptions to any generalization. Some animals do die slowly and in extended agony, even to the point of vocalization, as noted in 2 cases by Pienaar (1961). We have observed the same manifestations with some rabbits and monkeys. Some individuals show early signs of illness. To account adequately for exceptions to our generalization, more work needs to be done. The classical work of the Porton group (Smith et al, 1955) in identifying the toxin of *B. anthracis* forms the basis for our present work, the results of which make anthrax comparable to other toxin-producing organisms. It is perhaps most parallel to plague in which bacilli are disseminated throughout the body, but it is also similar to diphtheria and tetanus, diseases in which the causal organism is very localized.

Little more need be said about therapy of anthrax than what we have noted elsewhere (Lincoln et al, 1964a, b;

Klein et al, 1962, 1967): For a toxigenic disease the only known specific antidote is antitoxin (antiserum) which should be used in conjunction with bactericidal antibiotics (penicillin and streptomycin). The proper choice of treatment becomes more critical in the late stages of septicemia. Russian medical treatment of anthrax appears to make more use of antisera than does western medicine and uses a variety of drugs that would be expected to stimulate the cardiovascular system (Lebedev, 1961; Bunin, 1960). Our model on anthrax treatment and prophylaxis (Lincoln et al, 1964) adequately describes the interaction among the principal variables of this disease syndrome and is highly applicable to the suggested treatment of anthrax.

SUMMARY

A depression of the cortical electrical activity, observed on electroencephalograms, and subsequent respiratory failure occurred in rhesus monkeys dying of anthrax. The cardiac activity and respiratory rate did not change during the disease until, as evidenced by respiratory patterns, acute respiratory distress occurred. At this time typical anoxic responses were observed. White blood cell counts and hematocrit readings increased late in the course of disease and several hours after a septicemia had been observed. A terminal toxemia was demonstrable. The terminal responses were essentially identical to those that we have reported for rhesus monkeys and chimpanzees challenged with lethal amounts of sterile anthrax toxin. Anthrax is now visualized as a clinically new disease with many similarities to plague, diphtheria, tetanus, and other lethal toxigenic diseases.

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Reprinted from the Journal of Infectious Diseases
February, 1968, Vol 118
Pages 97–103